

**ANALYSIS OF BACTERIAL DIVERSITY AND
POLYHYDROXYALKANOATE SYNTHASE GENES
ASSOCIATED WITH SOME MARINE SPONGES**

AMELIA TAN SUET MAY

**MASTER OF SCIENCE
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**Thesis Submitted in Fulfilment of the Requirements for the Degree of
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DEDICATION

For my family and loved ones who inspired me to pursue my dreams.

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirements for the degree of Master of Science

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Main Supervisor : **Assoc. Prof. Dr. Kesaven Bhubalan, Ph.D.**
Co-Supervisor : **Prof. Dr. Amirul Al-Ashraf Abdullah, Ph.D.**
School/Institute/Centre : **School of Marine and Environmental Sciences**

Polyhydroxyalkanoate (PHA) is scientifically acclaimed as a naturally biosynthesised, biodegradable and biocompatible biopolymer that presents as an alternative to petroleum-based plastic. Recognised as a bacterial hotspot, the marine sponge is an acknowledged resource of novel natural products and genetic material, hence is a desirable target for PHA-producing bacteria or PHA-polymerising genes. However, due to the difficulty in cultivating sponge-associated bacteria plus the emergence of bioinformatics and metagenomics tools, the medically and biotechnologically appealing genes are increasingly exploited by bioengineering prior to biosynthesis. Therefore, in this study, the bacterial diversity and PHA synthase genes (*phaC*) of locally abundant yet underexplored sponges, explicitly *Aaptos aaptos* and *Xestospongia muta* from Pulau Bidong along with *A. aaptos* from Pulau Redang, were investigated. After metagenome extraction, bacterial diversity was analysed using culture-independent 16S ribosomal ribonucleic acid (16S rRNA) amplicon sequencing. Then, the *phaC* genes were screened, sequenced, identified and published in the GenBank database. Three *phaC* labelled 2, 2B and 1B were closely similar to the *phaC* of *Pseudomonas* sp., *Rhodocista* sp., and bacterium AR5-9d_16. Out of ninety-nine dominant genera, twenty-six PHA-producing genera were detected among the three bacterial communities. Geographical factors rather than host species notably influenced sponge-associated bacterial communities. The high-microbial-abundance

(HMA) sponge *X. muta* comprised the most diverse bacterial community, whereas the thus far uncharacterised bacterial diversity of sponge *A. aaptos* exhibited the attribute of an HMA sponge. The three identified *phaC* 2, 2B and 1B were submitted to GenBank under accession numbers MF437016, MF457754, and MF457753.

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**ANALISIS KEPELBAGAIAN BAKTERIA DAN
GEN PENGHASILAN POLIHIDROKSIALKANOAT
YANG BERSEKUTU DENGAN BEBERAPA SPAN LAUT**

AMELIA TAN SUET MAY

2018

Penyelia Utama : Assoc. Prof. Dr. Kesaven Bhubalan, Ph.D.

Penyelia Bersama : Prof. Dr. Amirul Al-Ashraf Abdullah, Ph.D.

Pusat Pengajian/Institut/Pusat: Pusat Pengajian Sains Marin dan Persekitaran

Polihidroksialkanoat (PHA) dikenali secara saintifik sebagai biopolimer yang disintesis secara semulajadi, boleh mengurai, dan serasi dengan badan manusia, serta merupakan alternatif kepada plastik berasaskan petroleum. Diakui sebagai titik panas bakteria, span laut diiktiraf sebagai sumber untuk produk semulajadi dan bahan genetik yang baru, justeru adalah sasaran yang wajar untuk bakteria penghasil PHA atau gen polimerisasi PHA. Walau bagaimanapun, disebabkan oleh kesulitan dalam pengkulturan bakteria span serta kemunculan alat bioinformatik dan metagenomik, gen yang menarik dari segi perubatan dan bioteknologi semakin dieksploitasi dengan kejuruteraan genetik sebelum biosintesis. Berikutan itu, dalam kajian ini, diversiti mikrob dan gen penghasil PHA (*phaC*) dari span tempatan yang banyak dijumpai tapi kurang dikaji, iaitu *Aaptos aaptos* dan *Xestospongia muta* dari Pulau Bidong dengan *A. aaptos* dari Pulau Redang, telah diterokai. Selepas pengekstrakan metagenom, diversiti mikrob dianalisis menggunakan penjujukan amplicon asid ribonukleik ribosoma 16S (16S rRNA) yang bebas kultur. Gen *phaC* telah ditapis, dijujuk, dikenalpasti, serta diterbit dalam pangkalan data GenBank. Tiga *phaC* berlabel 2, 2B dan 1B didapati sangat mirip dengan *phaC* dari *Pseudomonas* sp., *Rhodocista* sp., dan bakteria AR5-9d_16 yang belum dikultur. Dari sembilan puluh sembilan genus yang dominan, dua puluh enam genera penghasil PHA telah dikesan antara ketiga-tiga komuniti mikrob. Komuniti mikrob span secara khusus lebih

dipengaruhi oleh faktor geografi berbanding dengan spesies perumah. Span *X. muta* yang tinggi mikrob (HMA) mengandungi komuniti mikrob yang paling pelbagai, manakala komuniti mikrob *A. aaptos* yang hingga sekarang belum dicirikan menunjukkan komuniti mikrob span jenis HMA. Gen *phaC* 2, 2B dan 1B yang diidentifikasi telah diserahkan kepada GenBank dengan nombor akses MF437016, MF457754, dan MF457753.